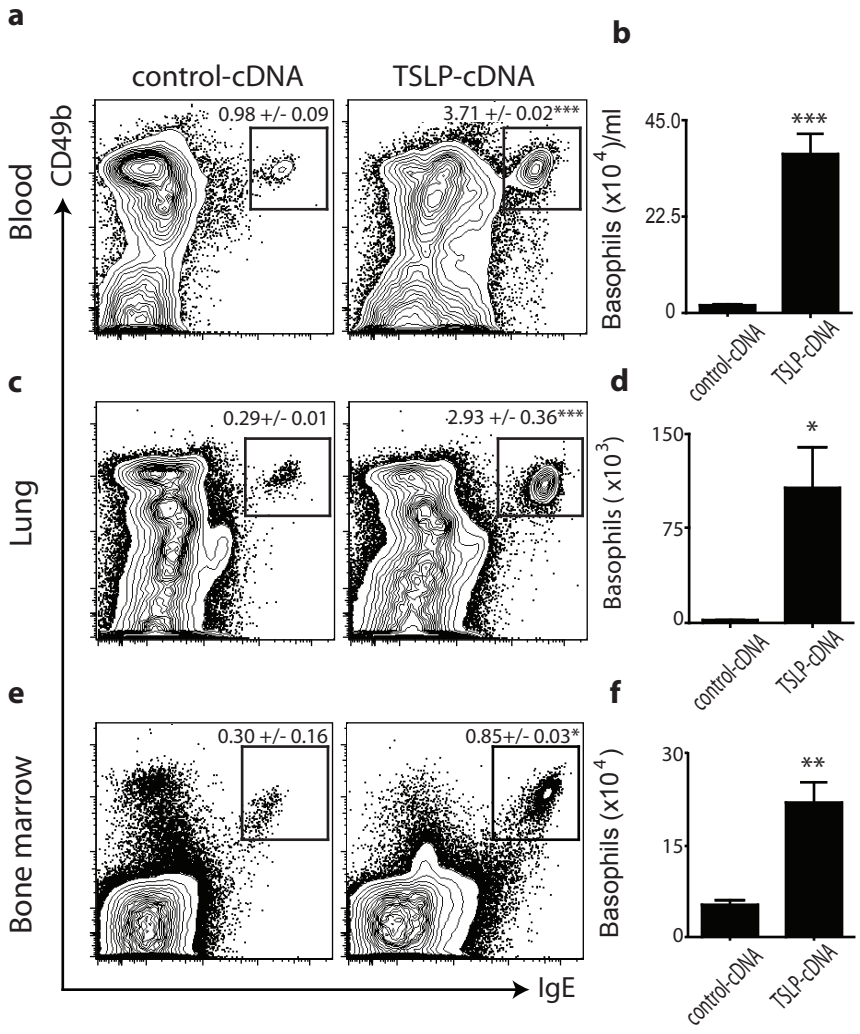
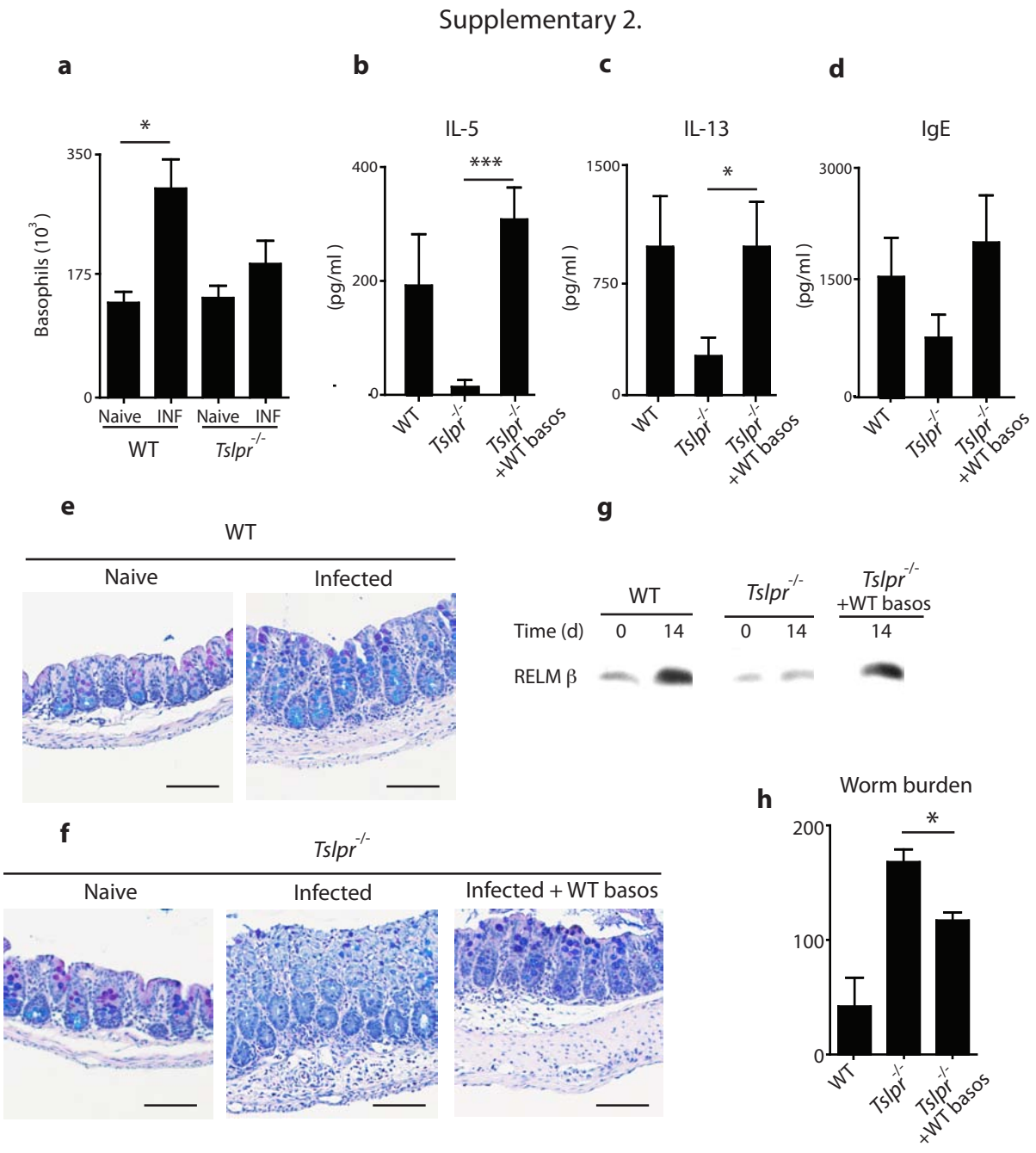
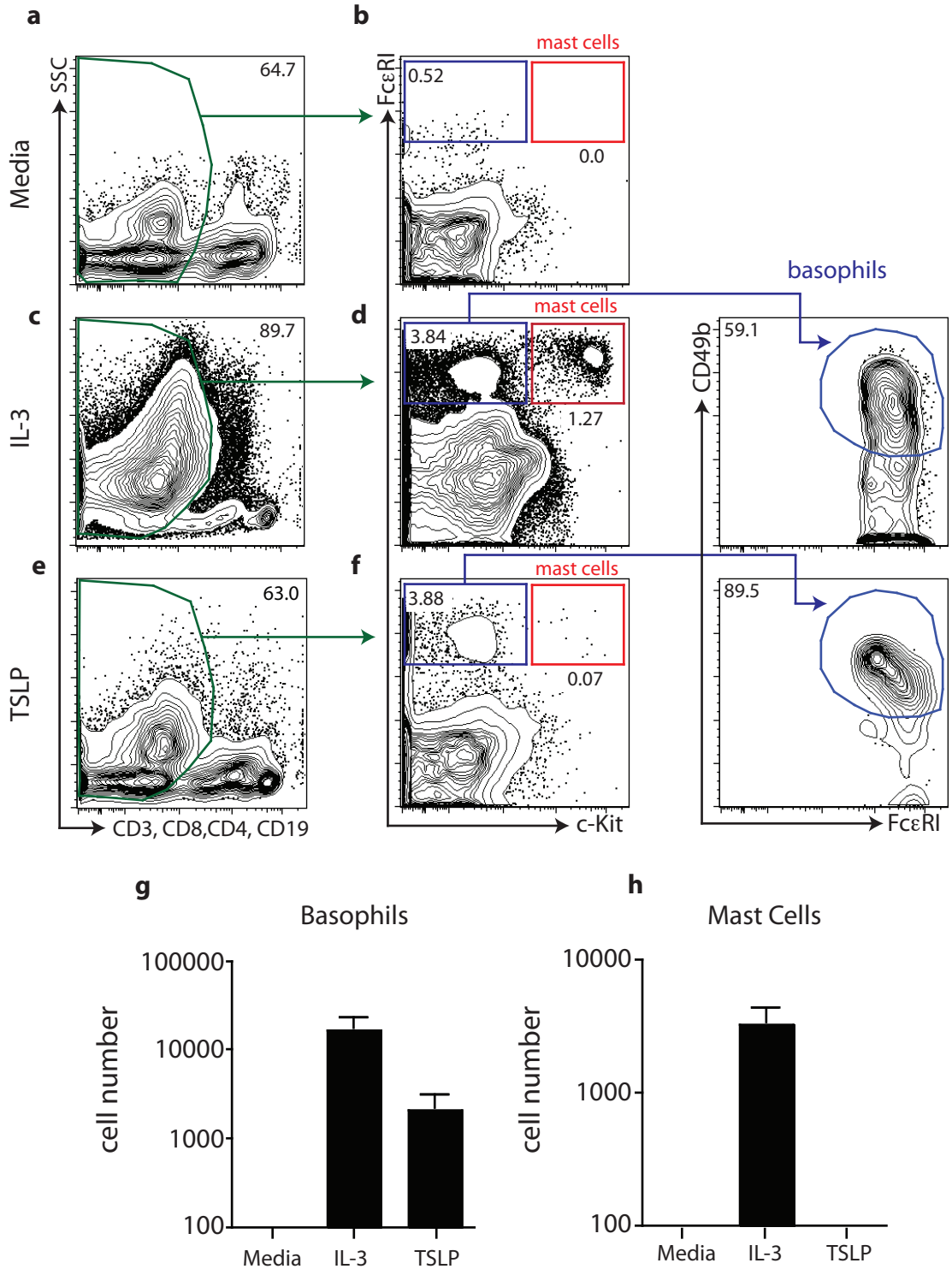


Supplementary Figure 1. TSLP promotes peripheral basophilia and Th2 cytokine responses. BALB/c mice were injected (i.v.) with a control DNA plasmid (control-cDNA) or a TSLP encoding plasmid (TSLP-cDNA), and **(a)** blood, **(c)** lungs and **(e)** bone marrow were harvested 20-21 days post-plasmid injection, processed and stained for basophils. Flow cytometry plots are gated on live, NBNT cells. Basophils were identified as NBNT, CD49b+, IgE+, FcεRI+ and c-Kit-. **(b,d,f)**, Total basophil numbers for each compartment were determined. Results are representative of two separate experiments of 5 mice per group. Statistical analysis was performed using a two-tailed students t test (*, $p<0.05$), (**, $p<0.01$), (***, $p<0.001$).



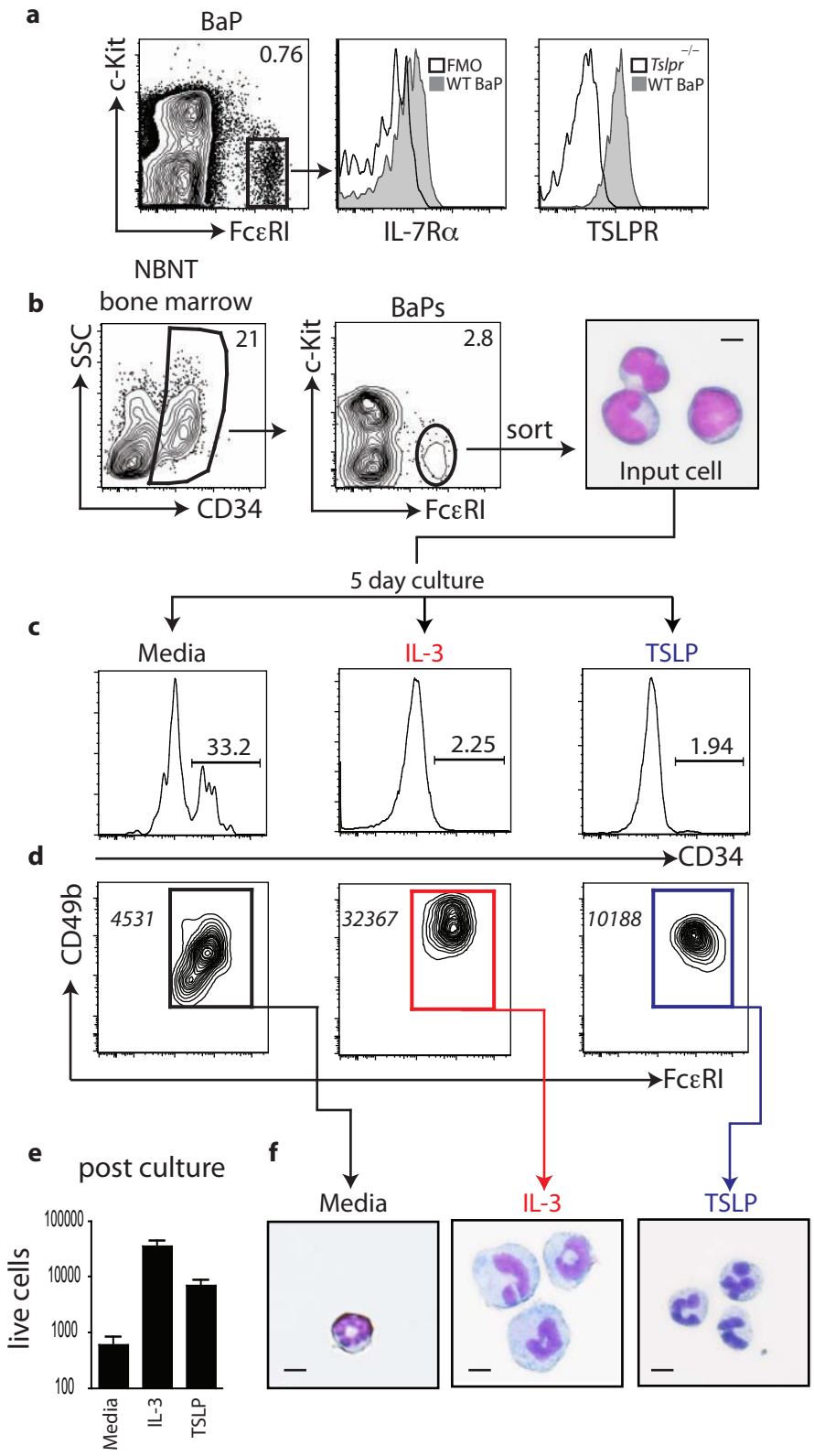
Supplementary Figure 2. Basophil-restricted TSLPR expression is sufficient to recover Th2 cell-dependent immunity to helminth infection. (a), WT or *Tslpr*-/- mice were infected with *Trichuris muris*, and splenic basophil populations were quantified on day 21 post-infection. Results are compiled from 3 separate experiments. **(b-h)**, WT or *Tslpr*-/- were infected with *T. muris*. One group of infected *Tslpr*-/- mice received 100-130 x 10^3 TSLPR-sufficient basophils (+WT basos). Mesenteric LN cells were isolated and stimulated with *T. muris* antigen collected from adult worms that were cultured for 4 hours in media. Antigen-specific production of **(b)** IL-5 and **(c)** IL-13 was determined by ELISA. **(d)**, Serum IgE levels, **(e, f)** goblet cell hyperplasia and intestinal mucin production (PAS staining), **(g)** luminal secretion of RELMβ and **(h)** worm burdens were determined on day 21 post-infection. **(a)**, Results are compiled from 3 separate experiments. **(b-h)**, Results are representative of two separate experiments. (WT mice n=10, *Tslpr*-/- n=10, *Tslpr*-/- mice that received 100-130 x 10^3 WT basophils n=6.) Scale bars, 100 µm. Statistical analysis was performed using a two-tailed students t test (*, $p<0.05$), (***,$p<0.001$). (IgE levels between *Tslpr*-/- and *Tslpr*-/- +WT basos, $p=0.083$).

Supplementary 3.



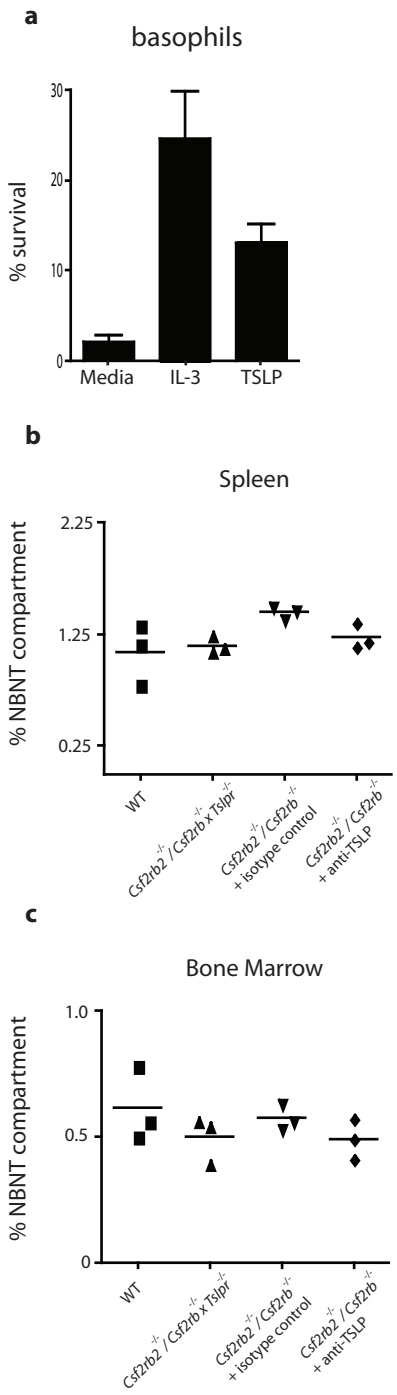
Supplementary Figure 3. TSLP selectively expands basophil but not mast cell populations from bone marrow-resident cells. Bone marrow was taken from WT mice and cultured in the presence of **(a)** media, **(c)** IL-3 or **(e)** TSLP for 5 days. **(b, d, f)**, Basophils were identified as NBNT, CD49b+, FcεRI+, c-Kit-. Mast cells were identified as NBNT, FcεRI+, c-Kit+. **(g)**, Total numbers of basophils and **(h)** mast cells were quantified on day 5 post-culture. (a), Results are representative of three separate experiments. (g, h), Cell numbers were compiled from three separate experiments.

Supplementary 4.



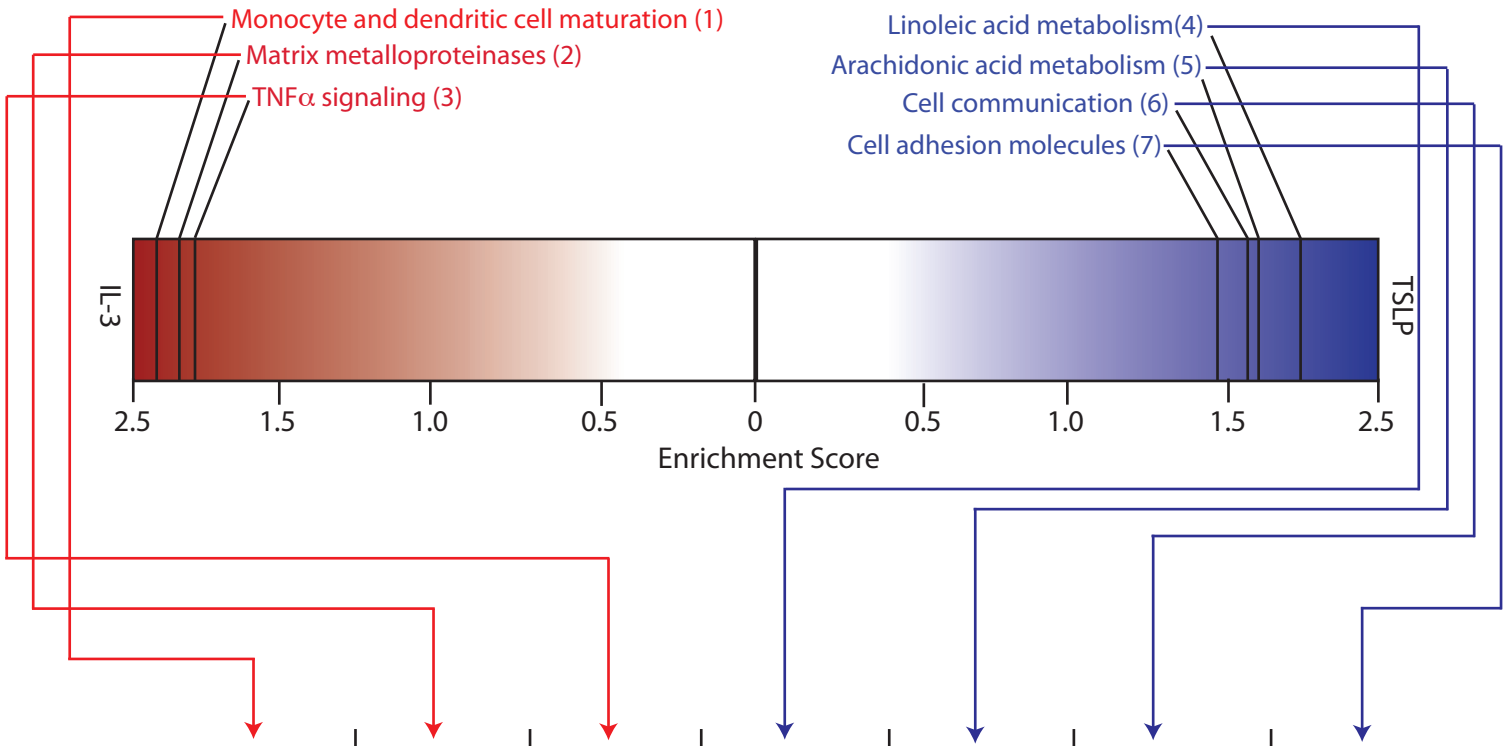
Supplementary Figure 4. TSLP acts directly on bone marrow-resident precursor cells. **(a)**, Basophil precursors from the bone marrow of WT or Tslpr-/- animals were identified using a BD FACS Aria II flow cytometer as NBNT, CD34+, FcεRI+, c-Kit-. Basophil precursor expression levels of IL-7Rα (shaded) were compared to fluorescence minus one (FMO) controls. Basophil precursor expression levels of TSLPR (shaded) were compared to basophil precursors in the bone marrow of Tslpr-/- mice. **(b)**, Basophil precursors (NBNT, CD34+, FcεRI+, c-Kit-) were sorted using a BD FACS Aria II flow cytometer and 10 x 10^3 cells were cultured for five days in the presence of media, IL-3 or TSLP. **(c,d)**, Cells were subsequently stained and analyzed using BD LSR II flow cytometer for FcεRI, CD49b and CD34 on day 5 post-culture. Italicized numbers represent MFI of CD49b staining. **(e)**, Total cells recovered on day 5 post-culture in the presence of media, IL-3 or TSLP. **(f)**, Cytology of cells after 5 day culture in the presence of media, IL-3 or TSLP was performed. **(a-d, f)**, Results are representative of at least three separate experiments. **(e)**, Results are compiled from three separate experiments. Scale bar, 10 μm.

Supplementary 5.



Supplementary Figure 5. IL-3 and TSLP promote mature basophil survival. (a), Basophils were sort-purified from the blood and spleens of WT mice and cultured over-night in media alone, IL-3 or TSLP. Basophil survival was measured flow cytometrically by exclusion of the viability dye 7-AAD. Results are compiled from three separate experiments. **(b)**, Spleen and **(c)** bone marrow cells were isolated from WT, *Csf2rb2-/-/ Csf2rb-/- x Tslpr-/-* mice or *Csfr2b2-/-/ Csf2rb-/-* mice treated with either an isotype control or neutralizing TSLP antibody over a 7 day period. Percentages of basophils in the NBNT compartment are illustrated. Basophils were identified as NBNT, CD49b+, Fcε RI+ and c-Kit-. Results are representative of two separate experiments of 3 mice per group.

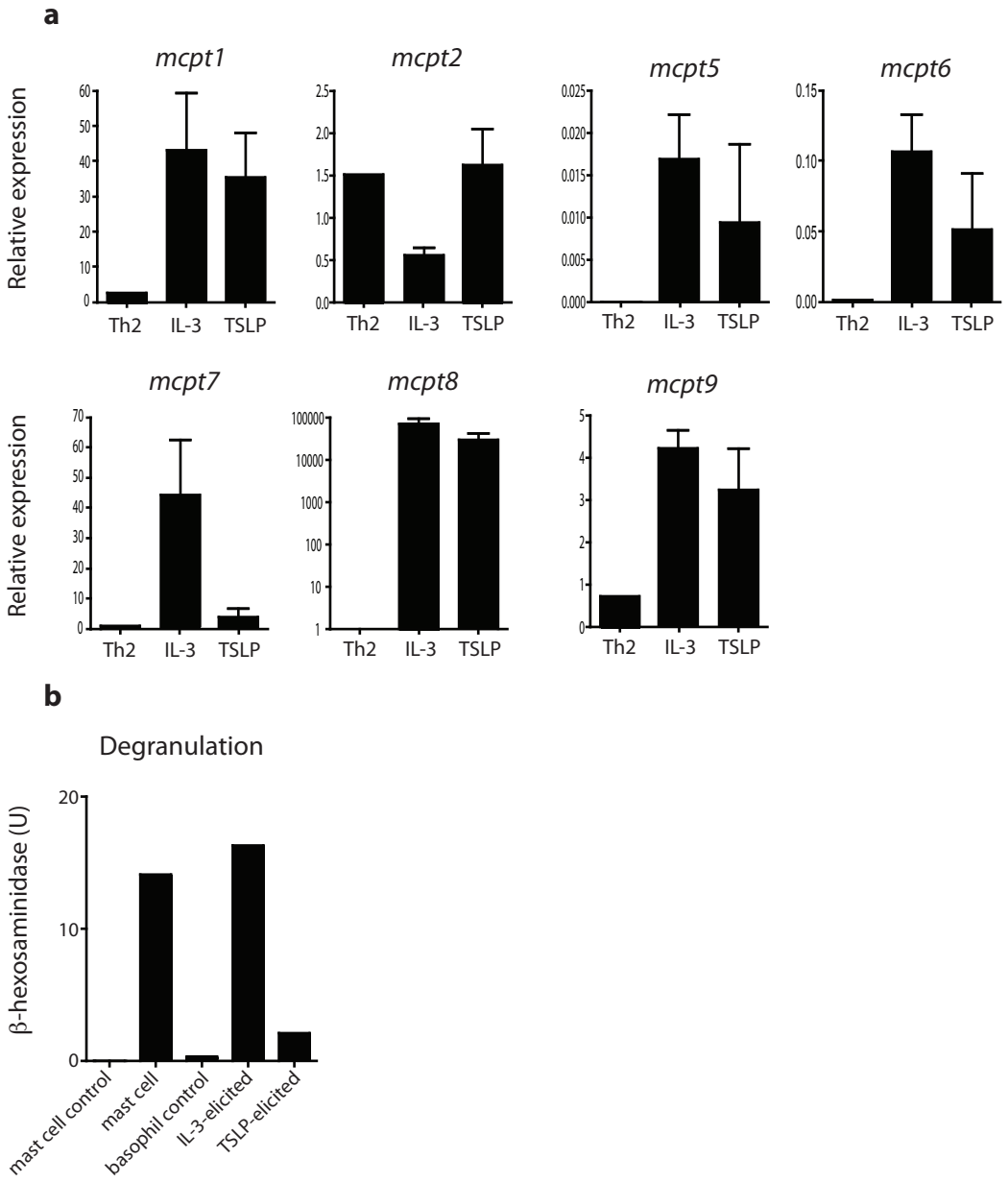
Supplementary 6.



Gene List (1)	Gene List (2)	Gene List (3)	Gene List (4)	Gene List (5)	Gene List (6)	Gene List (7)
C1QB (12260)	MMP13 (17386)	TSPAN3 (56434)	PLA2G3 (237625)	PTGDS (19215)	KRT39 (237934)	CD6 (12511)
MRC1 (17533)	MMP8 (17394)	CCNC (51813)	ALOX15 (11687)	DHRS4 (28200)	INA (226180)	NRXN1 (18189)
FABP4 (11770)	MMP9 (17395)	TGFBR3 (21814)	PLA2G10 (26565)	PLA2G1B (18778)	KRT77 (406220)	CD22 (12483)
CCL22 (20299)	MMP19 (58223)	PSMB8 (16913)	PLA2G12B (69836)	PTGIS (19223)	KRT17 (16667)	MPZ (17528)
LPL (16956)	TIMP3 (21859)	CCL2 (20296)	PLA2G5 (18784)	GPX2 (14776)	ITGB4 (192897)	CLDN3 (12739)
PPIC (19038)	MMP12 (17381)	PRDX1 (18477)	CYP2E1 (13106)	GPX5 (14780)	COL11A1 (12814)	PTPRC (19264)
GATM (67092)	TIMP1 (21857)	SOD2 (20656)		CBR3 (109857)	LAMA3 (16774)	CD80 (12519)
CD74 (16149)	TIMP2 (21858)	CXCL1 (14825)		PLA2G2D (18782)	KRT23 (94179)	CDH4 (12561)
CCL17 (20295)		ACLY (04112)		PTGES (64292)	LAMC2 (16782)	JAM3 (83964)
MMP12 (17381)		RNASEN (14000)		LTA4H (16993)	LAMB2 (16779)	CD4 (12504)
IARS (105148)		SF3A3 (75062)		PLA2G3 (237625)	COL2A1 (12824)	SDC2 (15529)
ACAA2 (52538)		PSMB7 (19177)		ALOX15 (11687)	LMNB2 (16907)	PDCD1 (18566)
CD86 (12524)		ITGB2 (16414)		GPX6 (75512)	KRT78 (332131)	PVRL2 (19294)
CCR7 (12775)		KIT (16590)		PTGS2 (19225)	GJB5 (14622)	PVRL1 (58235)
SPP1 (20750)		NFKBIA (18035)		PLA2G10 (26565)	COL3A1 (12825)	ITGA4 (16401)
GDF15 (23886)				PLA2G12B (69836)	KRT6B (16688)	JAM2 (67374)
TRIB2 (217410)				PLA2G5 (18784)	DSC1 (13505)	NRXN3 (18191)
PDLIM4 (30794)				CYP2E1 (13106)	COL6A1 (12833)	CLDN5 (12741)
SLA (20491)					COL4A2 (12827)	CDH5 (12562)
HSD11B1 (15483)					KRT72 (105866)	NLGN2 (216856)

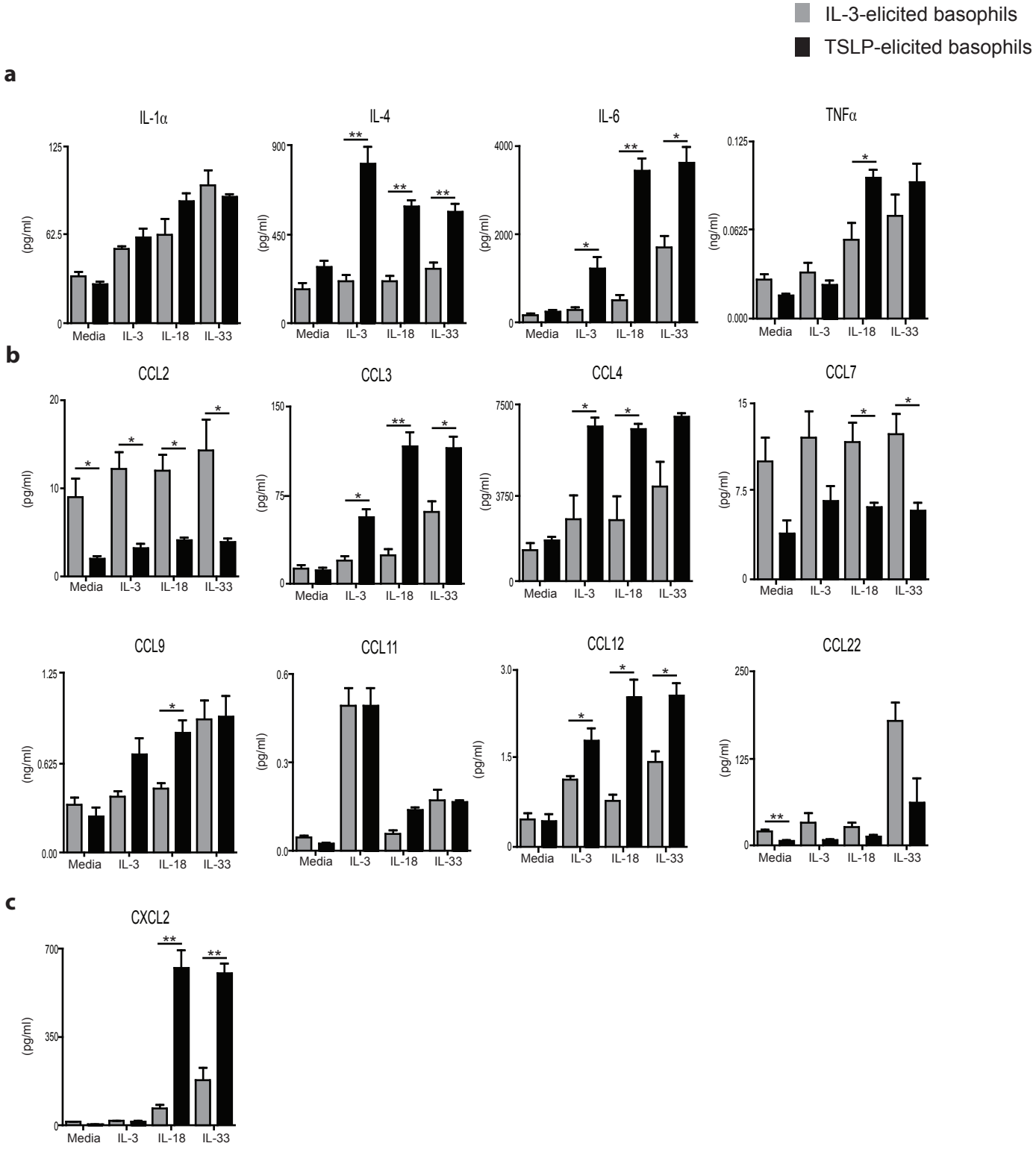
Supplementary Figure 6. Heterogeneity of basophil populations revealed by genome-wide transcriptional profiling. Bone marrow was taken from WT mice and cultured in the presence of IL-3 or TSLP for 5 days. NBNT, CD49b+, FcεRI+, c-Kit- basophils were sorted, RNA was extracted and samples were analyzed by microarray. Gene set enrichment analysis (GSEA) was performed with software available at http://www.broadinstitute.org/gsea/index.jsp and illustrated enrichment of coordinated gene sets that correspond to previously defined biological processes. List of genes for each biological process numbered in (Fig. 4c) that contribute to the enrichment score as outlined at http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html. EntrezGene accession numbers for each gene are in parenthesis.

Supplementary 7.



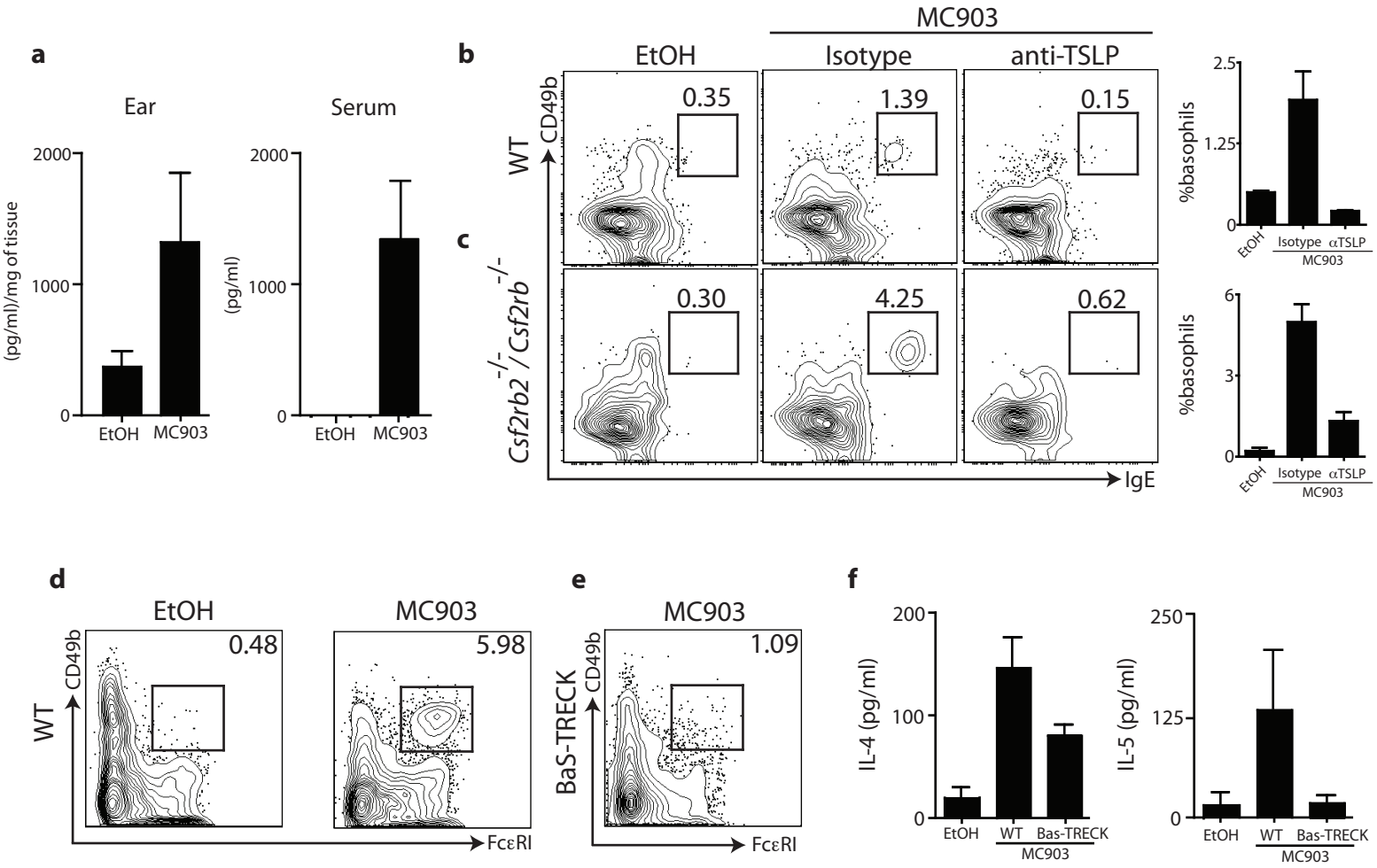
Supplementary Figure 7. IL-3- and TSLP-elicited basophils exhibit phenotypic and functional heterogeneity. **(a)**, Bone marrow was taken from WT mice and cultured in the presence of IL-3 or TSLP for 5 days. NBNT, CD49b+, Fcε RI+, c-Kit- basophils were sort-purified, and RNA was isolated. Th2 cells were isolated from the mesenteric LN of *T. muris* infected mice on day 21 post-infection and used as a negative expression control. Relative gene expression was normalized to bone marrow-derived mast cells that were grown in IL-3 and stem cell factor (SCF) for 8 weeks. Real time PCR was performed on four biological replicates of basophils. **(b)**, Bone marrow was taken from WT mice and cultured in the presence of IL-3 or TSLP for 5 days. NBNT, CD49b+, CD200R+, c-Kit- basophils were sorted and rested for 24 hours. Bone marrow derived mast cells were grown in the presence of IL-3 and SCF for 8 weeks. Cells were incubated with 1 μg/ml of anti-dinitrophenol (anti-DNP) IgE, washed, and stimulated with 100 ng/ml DNP-conjugated human serum albumin. Supernatants were tested for β-hexosamindase by incubating with p-nitrophenyl-N-acetyl-ß-D-glucosamide and measuring absorbance at 405 nm.

Supplementary 8.



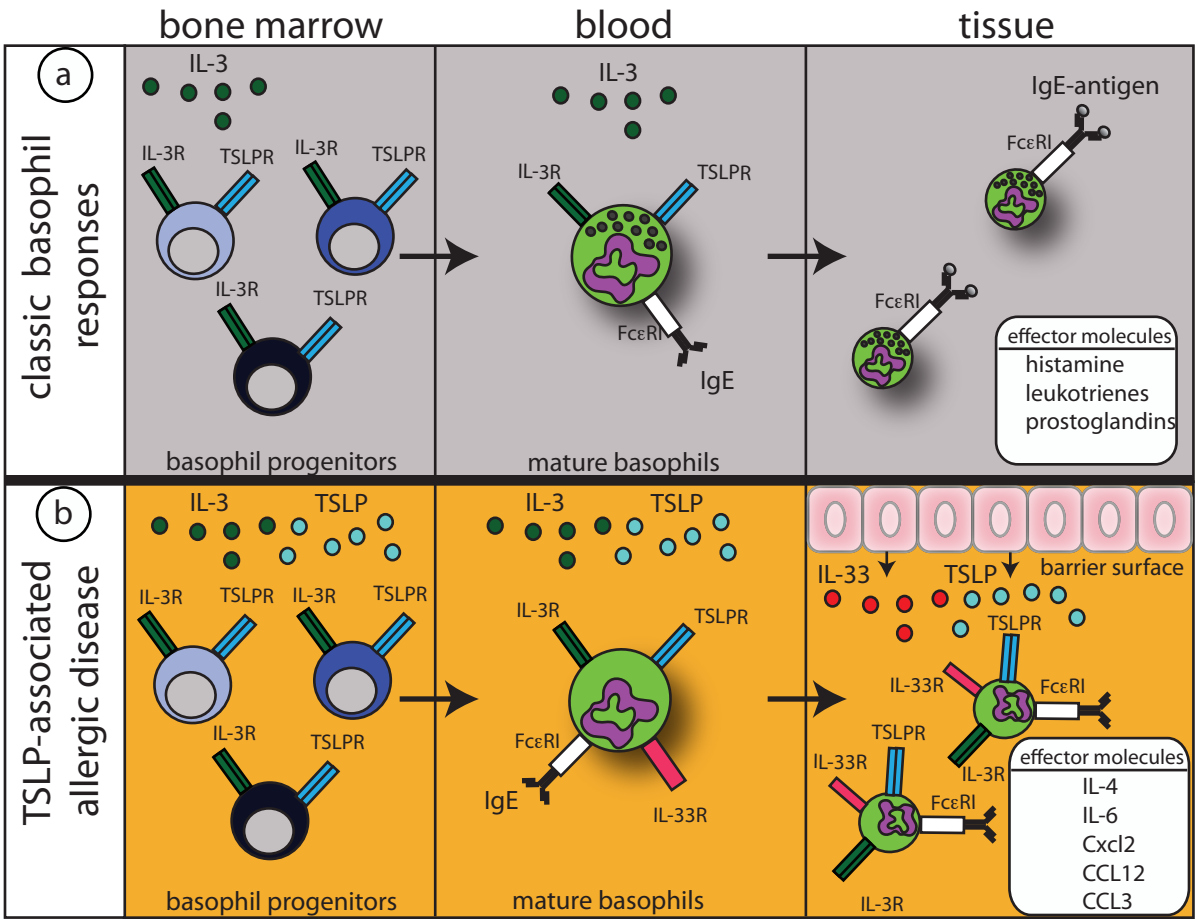
Supplementary Figure 8. IL-3- and TSLP-elicited basophils exhibit differential capabilities to respond to IL-3, IL-18 and IL-33. Bone marrow was taken from WT mice and cultured in the presence of IL-3 or TSLP for 5 days. NBNT, CD49b+, Fcε RI+, c-Kit- IL-3-elicited basophils (gray bars) or TSLP-elicited basophils (black bars) were sorted and stimulated for 12 hours with media alone, IL-3, IL-18 or IL-33. Cell free supernatants were removed and assayed for **(a)** IL-1α, IL-4, IL-6, TNFα, **(b)**CCL2, CCL3, CCL4, CCL7, CCL9, CCL11, CCL12, CCL22 and **(c)** CXCL2 by multi-analyte panel (Rodent v2.0), (http://www.rulesbasedmedicine.com/). Results from three biological replicates are shown. Statistical analysis was performed using a two-tailed students t test (*, $p<0.05$), (**, $p<0.01$).

Supplementary 9.



Supplementary Figure 9. TSLP regulates *in vivo* basophil responses independently of IL-3. **(a)**, WT mice were treated topically on the ear with ethanol (EtOH) or MC903 for 3 days, and local production of TSLP in the ear and serum levels of TSLP were determined by ELISA. **(b)**, WT mice or **(c)**, *Csfr2b2-/-/Csf2rb-/-* mice were treated topically on the ear with ethanol or MC903 for four days. NBNT, CD49b+, FcεRI+, c-Kit- basophil responses in the ear were determined after treatment with isotype or anti-TSLP antibody. **(a)**, results are representative of three separate experiments of 3 mice per group. (b,c), results are representative of two separate experiments of 3 mice per group. **(d)**, WT mice were treated topically on the ear with ethanol or MC903 for 7 days. **(e)**, BaS-TRECK mice were treated topically on the ear with ethanol or MC903 for 7 days. All mice were treated with diphtheria toxin (i.p.). NBNT, CD49b+, FcεRI+, c-Kit- basophil responses were determined on day 7 post-MC903. **(f)**, Draining LNs were stimulated with anti-CD3 and anti-CD28 for 48 hours, and IL-4 and IL-5 production were quantified by ELISA. Results are representative of two separate experiments. (Ethanol n=6, MC903 n =6, Bas-TREK n=5).

Supplementary 10.



Supplemental Figure 10. Proposed model of TSLP-dependent basophil development and function. **(a)**, IL-3-dependent development of classical basophils occurs in the bone marrow. IL-3 promotes the proliferation, maturation and subsequent exit of basophils into the periphery. Once in the periphery, IL-3 promotes mature basophil survival and surface-bound IgE is loaded via FcεRI binding. In the context of an inflammatory response, antigen-specific IgE+ basophils migrate into inflamed tissues. Antigen binds surface-bound IgE triggering FcεRI crosslinking, degranulation and production of histamines, leukotrienes and prostaglandins. **(b)** Basophil maturation in individuals suffering from TSLP-associated allergic diseases occurs when IL-3 binds the IL-3R and TSLP binds the TSLPR on basophil precursors, promoting their proliferation, maturation and subsequent exit into the periphery. Once in the periphery, basophil survival is enhanced via IL-3-IL-3R signaling, while TSLP-TSLPR signaling induces surface expression of the IL-33R. Although surface-bound IgE is loaded via FcεRI, TSLP-activated basophils do not degranulate in response to FcεRI crosslinking. In the context of inflammation at barrier surfaces, TSLP-elicited basophils migrate to inflamed tissue where they encounter IL-33, triggering their exaggerated production of IL-4, IL-6, CxCl2, CCL12 and CCL3.